Claims

It is claimed:

1. An enzymatically active variant of a precursor phenol oxidizing enzyme, said precursor being derived from a Stachybotrys species.

2. An enzymatically active variant of a precursor phenol oxidizing enzyme, which precursor phenol oxidizing enzyme has at least 68% identity to the amino acid sequence as disclosed in SEQ ID NO:2, said variant comprising a sequence that differs from that of said precursor in at least one of the positions 48, 67, 70, 76, 83, 98, 115, 119, 134, 171, 175, 177, 179, 188, 236, 246, 253, 254, 269, 272, 296, 302, 308, 318, 329, 331, 346, 348, 349, 365, 390, 391, 394, 404, 415, 423, 425, 428, 434, 465, 479, 481, 483, 499, 550, 562, 570, and 573, or sequence positions corresponding thereto.

3. The phenol oxidizing enzyme variant of claim 2, wherein said variant comprises a sequence that differs from that of said precursor in at least one of the positions 254, 272, 346, 348, 394, and 425, or sequence positions corresponding thereto.

4. The phenol oxidizing enzyme variant of claim 2 including an amino acid substitution at one or more of the positions or position sets: 76/254/302; 76/254/302/188; 76/254/302/394/425; 119/254/329; 119/254/390; 119/254/415; 171/254/346; 236/254; 254; 254/272; 254/302/346/348; 254/346/348; 254/394; 254/550; and 394/425.

5. The phenol oxidizing enzyme variant of claim 2 having at least one amino acid substitution or substitution set selected from:

N391S
G115S
D562G
D394N/V425M
V134I/H177Y
L499F
M254F

M254F/L499F
M98L/M254F
L76W/M254F
M254F/F349Y
H175V
H177V
L76W/M254F/E302V
M254F/D394N/V425M
L76W/M254F/E302V/D394N/V425M
M254F/A296S
M254F/W318Y
M254F/L48Y
M254F/R83K
M254F/M188F
M254F/Q246H
M254F/S331T
M254F/V483T
M254F/R67T
V119L/M254F/N70V
M254F/N70V
M254F/D308S
M254F/E365T
M254F/S415A
M254F/R423A
M254F/D428G
M254F/R434E
M254F/E465M
M254F/A479G
M254F/N550A
P253A
V119L/M254F/A269M
M254F/A269M
V119L/M254F/G329N
M254F/G329N
M254F/S331A
M254F/E346V/E348Q
V119L/M254F/E346V
M254F/E346V
V119L/M254F/A390P
M254F/A390P
M254F/N404T
V119L/M254F/S415L
M254F/S415L
M254F/R481G
M254F/A573N
M254F/A573N/F570L
M254F/L76W/E302V/M188K

M254N
M254L
M254A
M254I
M254E
M254S
M254H
M254V
M254T
M254P
M254G
M254K
M254C
M254F/D394G
M254F/D394V
M254F/D394S
M254F/D394H
M254F/D394P
M254F/D394Y
M254F/D394W
M254F/D394N
M254F/M179F
M254F/M179V
M254F/M179P
M254F/M179G
M254F/M179E
M254F/M179L
M254F/I181D
M254F/S180F/I181L
M254F/E346V/E302I
M254F/E346V/E302K
M254F/E346V/E348Q/E302F
M254F/E346V/E348Q/E302A
M254F/E346V/E348Q/E302L
M254F/E346V/E302C
M254F/E346V/E302V
M254F/E346V/M171T
M254F/E346V/E348Q/M171P
M254F/E346V/E348Q/M171L
M254F/E346V/M171Y
M254F/ E346V/E348Q/M171V
M254F/ E346V/M171S
M254F/E346V/M171R
M254F/E346V/E348Q/M171F
M254F/E346V/ M171K
M254F/E346V/E348Q/M171Q
M254F/E346V/E348Q/M171N

M254F/E346V/E348Q/M171N/L172H
M254F/S272L
M254F/E236K
M254F/E346V/E348Q/M188K/D394W/S272L/E236K
M254F/E346V/E348Q/M188K/D394W/E236Q
M254F/E346V/E348Q/M188K/D394W/E236K
M254F/E346V/E348Q/M188K/D394W/E236D
M254F/E346V/E348Q/M188K/D394W/E236A
M188K/M254F/E346V/E348Q/D394W

6. The phenol oxidizing enzyme variant of any one of claims 2, 3, 4, or 5, wherein said precursor phenol oxidizing enzyme has at least 80% identity, and preferably at least 85% identity, to the amino acid sequence disclosed in SEQ ID NO:2.

7. The phenol oxidizing enzyme variant of any one of claims 2, 3, 4, or 5, wherein said precursor phenol oxidizing enzyme has at least 90% identity, and preferably at least 95% identity, to the amino acid sequence disclosed in SEQ ID NO:2.

8. The phenol oxidizing enzyme variant of claim 7, wherein said precursor phenol oxidizing enzyme has the amino acid sequence disclosed in SEQ ID NO:2.

9. The phenol oxidizing enzyme variant of claim 2, wherein said phenol oxidizing enzyme variant has increased phenol oxidizing activity at high pH as compared to said precursor phenol oxidizing enzyme.

10. The phenol oxidizing enzyme variant of claim 9, having a pH optimum of at least 8, and preferably at least 9.

11. The phenol oxidizing enzyme variant of claim 2, wherein said precursor is obtainable from a Stachybotrys species, preferably Stachybotrys chartarum.

12. An isolated polynucleotide encoding the variant of claim 2.

13. An expression vector comprising the polynucleotide of claim 12.

14. A host cell comprising the expression vector of claim 13.

15. The host cell of claim 14, wherein said host cell is a filamentous fungus.

16. The host cell of claim 15, wherein said fungus is an Aspergillus species or a Trichoderma species.

17. An isolated protein having phenol oxidizing activity, which comprises an amino acid sequence having at least 68% and less than 100% identity to the amino acid sequence disclosed in SEQ ID NO:2, and which differs from said SEQ ID NO:2 sequence in at least one of the positions 48, 67, 70, 76, 83, 98, 115, 119, 134, 171, 175, 177, 179, 188, 236, 246, 253, 254, 269, 272, 296, 302, 308, 318, 329, 331, 346, 348, 349, 365, 390, 391, 394, 404, 415, 423, 425, 428, 434, 465, 479, 481, 483, 499, 550, 562, 570, and 573, or sequence positions corresponding thereto.

18. A method for obtaining a phenol oxidizing enzyme variant derived from a Stachybotrys species, said variant having at least one altered property relative to a precursor phenol oxidizing enzyme, which comprises the steps of:

mutagenizing a gene encoding said precursor phenol oxidizing enzyme, which precursor enzyme comprises an amino acid sequence having at least 68% identity to the amino acid sequence shown in SEQ ID NO:2;

introducing the mutant gene into a host strain whereby a transformed host strain is obtained;

growing said transformed host whereby said mutant gene is expressed and a phenol oxidizing enzyme variant, differing from said precursor enzyme by one or more amino acid substitutions, is identified by recovering said variant and screening it for increased phenol oxidizing activity and/or increased pH optimum.

19. The method of claim 18, wherein said one or more amino acid substitutions correspond to amino acid positions selected from the group consisting of 48, 67, 70, 76, 83, 98, 115, 119, 134, 171, 175, 177, 179, 188, 236, 246, 253, 254, 269, 272, 296, 302, 308, 318, 329, 331, 346, 348, 349, 365, 390, 391, 394, 404, 415, 423, 425, 428, 434, 465, 479, 481, 483, 499, 550, 562, 570, and 573 of said SEQ ID NO:2 sequence.

20. The method of claim 18, wherein said mutagenized gene is a cloned Stachybotrys gene, preferably a cloned Stachybotrys chartarum gene, or a cloned gene capable of hybridizing to such a Stachybotrys gene under conditions of intermediate to high stringency.

21. A method for producing a variant of a precursor phenol oxidizing enzyme, which precursor enzyme comprises an amino acid sequence having at least 68% identity to the amino acid sequence shown in SEQ ID NO:2; said method comprising the steps of:

a) culturing a host cell comprising a polynucleotide encoding said variant, wherein said variant differs from said precursor sequence in at least one of the positions 48, 67, 70, 76, 83, 98, 115, 119, 134, 171, 175, 177, 179, 188, 236, 246, 253, 254, 269, 272, 296, 302, 308, 318, 329, 331, 346, 348, 349, 365, 390, 391, 394, 404, 415, 423, 425, 428, 434, 465, 479, 481, 483, 499, 550, 562, 570, and 573, or sequence positions corresponding thereto, under conditions suitable for the production of said variant; and

(b) optionally recovering said variant produced.

22. A method for producing a host cell comprising a polynucleotide encoding a variant of a precursor phenol oxidizing enzyme, which precursor enzyme comprises an amino acid sequence having at least 68% identity to the amino acid sequence shown in SEQ ID NO:2; said method comprising the steps of:

(a) obtaining a polynucleotide encoding said variant, wherein said variant differs from said precursor sequence in at least one of the positions 48, 67, 70, 76, 83, 98, 115, 119, 134, 171, 175, 177, 179, 188, 236, 246, 253, 254, 269, 272, 296, 302, 308, 318, 329, 331, 346, 348, 349, 365, 390, 391, 394, 404, 415, 423, 425, 428, 434, 465, 479, 481, 483, 499, 550, 562, 570, and 573, or sequence positions corresponding thereto;

(b) introducing said polynucleotide into said host cell; and

(c) growing said host cell under conditions suitable for the production of said variant.

23. The method of claim 22, wherein said host cell is a filamentous fungus.

24. The host cell of claim 23, wherein said fungus is a Aspergillus species or a Trichoderma species.